

# Characterization and Cardiovascular Effects of (13S)-9 $\alpha$ ,13 $\alpha$ -epoxylabda-6 $\beta$ (19),15(14)diol Dilactone, a Diterpenoid Isolated from *Leonotis leonurus*

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## ABSTRACT

A new diterpenoid, (13S)-9 $\alpha$ ,13 $\alpha$ -epoxylabda-6 $\beta$ (19),15(14)diol dilactone (**1**), was isolated from *Leonotis leonurus* and the structure determined *via* NMR analysis. The compound causes significant changes in blood pressure of anaesthetized normotensive rats and exhibits a negative chronotropic effect.

## KEYWORDS

*Leonotis leonurus*, diterpenoid, NMR analysis, chronotropic effect.

## 1. Introduction

The pharmacological evaluation of plants used in traditional medicines represents a promising approach to the discovery of new drugs. However, only a small number of the plants used extensively in traditional medicine in South Africa have been fully studied to assess their purported pharmacological effects.<sup>1</sup> *Leonotis leonurus* (Watt 1962) (Lamiaceae) is a shrub indigenous to the southern tip of Africa and traditionally the leaves have been smoked to relieve epileptic fits, leaf infusions or decoctions used externally to treat boils, eczema, skin diseases, itching and muscular cramps, and internally to treat influenza, headaches, jaundice, bronchitis, coughs and high blood pressure.<sup>2–4</sup> Our particular interest in *L. leonurus* lies in its cardiovascular effects and while studies relating to this topic have been undertaken on crude aqueous and methanol extracts of the plant,<sup>5–8</sup> no investigation of the cardiovascular effects of any single pure compound has been carried out, in spite of the fact that many have been isolated.<sup>9–13</sup> All isolated compounds to date have been diterpenoids and with respect to the cardiovascular system, these types of compounds have been shown to relax vascular smooth muscles and decrease heart rate in isolated arteries and anaesthetized rats respectively.<sup>14–20</sup>

## 2. Results and Discussion

Our ongoing investigation into the phytochemistry of *L. leonurus* has led to the isolation of a further new diterpenoid (**1**) from the methanol extract of the plant. The high-resolution electron impact mass spectrum (HREIMS) indicated a [M+H]<sup>+</sup> ion at *m/z* 349.2009, which correlates with the molecular formula C<sub>20</sub>H<sub>29</sub>O<sub>5</sub> (calcd. for C<sub>20</sub>H<sub>29</sub>O<sub>5</sub> 349.2015). From the <sup>1</sup>H NMR spectrum the diastereotopic protons in the  $\delta$  1 to 2.5 ppm region and the doublet of doublets at  $\delta$  4.70 ppm indicated that the new molecule was most likely a diterpenoid similar to those previously isolated from the plant (see Fig. 1). The <sup>13</sup>C NMR spectrum indicated the presence of 20 unique carbon atoms with the DEPT spectrum showing there to be three methyl, eight CH<sub>2</sub> and three CH groups. Based on the distinctive chemical shifts of two of the

remaining six quaternary carbons at  $\delta$  83.6 and 93.5 ppm the presence of two-spiro fused five-membered rings was very likely. In addition, two carbonyl moieties ( $\delta$  183.8 and 171.4 ppm) could be identified. At this point it was ascertained from the above information that the molecule was similar to a diterpenoid simply known as compound X, that had previously been isolated from *L. leonurus*,<sup>10,12</sup> and which assisted in the deduction of the structure of **1** for this new diterpenoid. Using HSQC, HMBC and COSY NMR spectra it was confirmed that **1** and compound X are positional isomers of one another with the lactone carbonyl being at C-14 in **1** rather than at C-15 as in compound X. This is clear from the presence of the ethano carbons at C-15 and C-16 in **1** (coupling seen in the <sup>1</sup>H and COSY NMR spectra) as opposed to compound X which has two isolated CH<sub>2</sub> groups at C-14 and C-16. The relative stereochemistry of **1** is consistent with that of other diterpenoids isolated from *L. leonurus*,<sup>9–13</sup> as evidenced from the scalar couplings measured in the <sup>1</sup>H NMR spectrum and also from correlations observed in the 2D NOESY spectrum collected (see Fig. 2). This is the first time to our knowledge that diterpenoid **1** has been isolated from *L. leonurus* or any other plant.

Diterpenoid **1** was administered intravenously to anaesthetized normotensive male Wistar rats in order to evaluate its cardiovascular activity. Results indicate dose-dependent changes in blood pressure and heart rate (HR) in the rats. Interestingly, on the one hand, the 0.5, 1.0 and 2.0 mg kg<sup>-1</sup> doses produced dose-dependent decreases in systolic pressure (SP), diastolic pressure (DP) and mean arterial pressure (MAP), all of which were statistically significant (Fig. 3). On the other hand, administration of the higher 3.0, 4.0 and 5.0 mg kg<sup>-1</sup> doses produced dose-dependent increases in SP, DP and MAP which were also statistically significant (Fig. 3). All doses induced significant dose-dependent decreases in HR in the animals (Fig. 4).

The decrease in blood pressure at the lower doses is similar to the effects observed by Mugabo *et al.* and Ojewole in anaesthetized normotensive animals using crude aqueous extracts of the plant,<sup>5,6</sup> while the increase in blood pressure at higher doses

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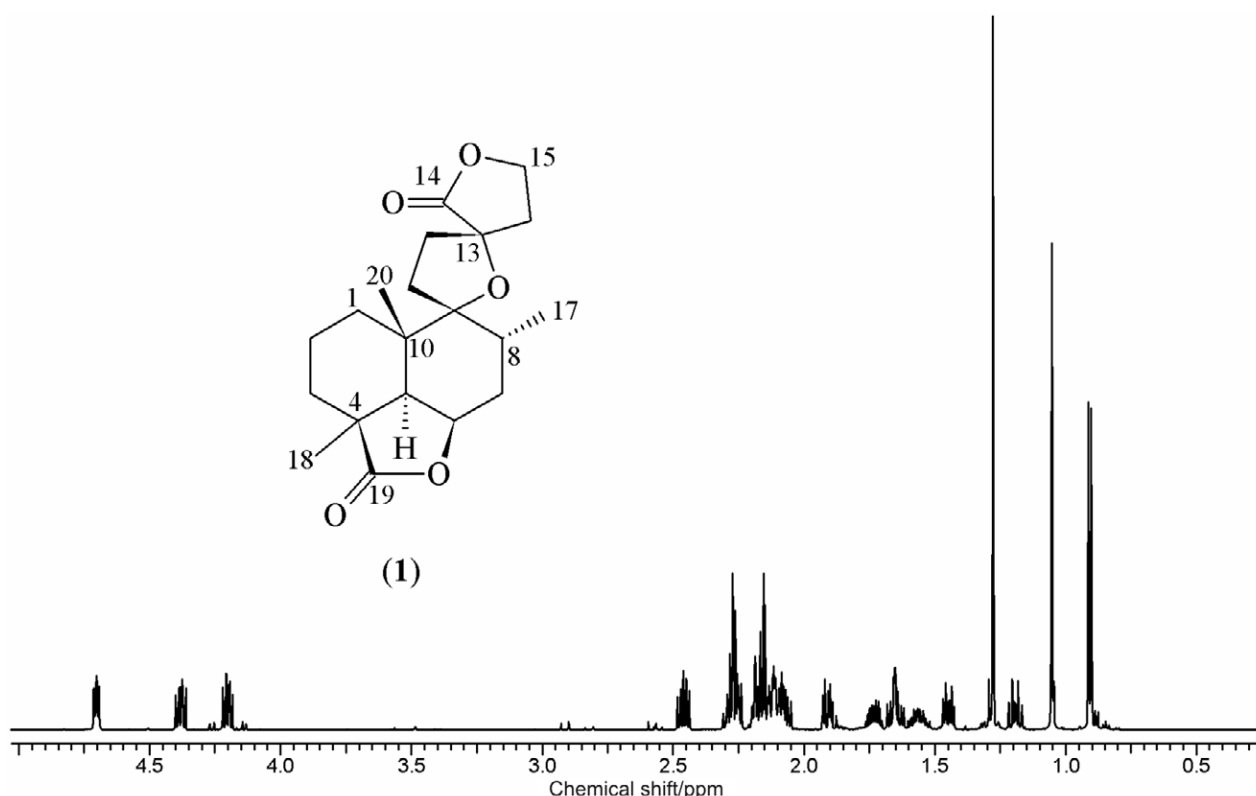


Figure 1 Structure and  $^1\text{H}$  NMR spectrum of (13*S*)-9 $\alpha$ ,13 $\alpha$ -epoxylabda-6 $\beta$ (19),15(14)diol dilactone (1).

(3.0–5.0 mg kg $^{-1}$ ) was similar to that noted in earlier experiments using crude aqueous extracts of the leaves only.<sup>8</sup> The effect on HR was similar to that observed by Obikeze *et al.* and Ojewole with the crude aqueous extract, but was opposite to the increase in HR observed by Obikeze *et al.* with methanol fractions of the leaves.<sup>5,7,8</sup> The differences in the effects observed by the different researchers with the plant may be explained by the likely presence of more than one cardio-active compound in the crude plant extracts. Diterpenoids isolated from other plants have also been shown to exhibit varying effects on the cardiovascular system.<sup>16,17</sup> Most diterpenoids assayed for cardiovascular activity seem to have singular effects, and those that have been shown to exhibit bi-phasic effects are thought to do so mainly *via* baroreceptor reflex mechanisms in the animals.<sup>18,19</sup> The decrease in blood pressure observed at the low doses of diterpenoid 1 may be due to vasodilation, which has been reported with the crude aqueous extract and with many diterpenes assayed for cardiovascular activity.<sup>5,16,20</sup> Activation of the baroreceptor reflex mechanism may explain the observed increase in BP at the higher doses of diterpenoid 1. A similar scenario involving

the activation of the baroreceptor reflex has been reported in anaesthetized rats administered with a labdenic diterpene Labd-8 (17)-en-15-oic acid by Lahlou and co-workers.<sup>18</sup>

Diterpenoid 1 exhibited a negative chronotropic effect on the heart, and diterpenoids have been shown to exhibit different effects on heart rate through the mechanisms of calcium channel antagonism, adenylate cyclase activation, Na $^+$ /K $^+$  channel blockade and the release of nitric oxide.<sup>16,19–21</sup> Our results suggest that the cardiovascular effects of diterpenoid 1 are mediated either by the blockade of  $\beta_1$  receptors in the heart, or the inhibition of Ca $^{2+}$ , Na $^+$  and/or K $^+$  channels in the heart. Further studies are, however, required to elucidate the exact mechanism of action of diterpenoid 1.

### 3. Experimental

Optical rotations were measured with a Bellingham & Stanley ADP 220 polarimeter (Tunbridge Wells, England). IR spectra were obtained using a Perkin-Elmer Paragon 1000 PC (Waltham, MA, USA).  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^1\text{H}$ - $^1\text{H}$  COSY, HSQC, HMBC, DEPT and NOESY NMR spectra were collected on a Varian UnityInova 600 NMR spectrometer (Palo Alto, CA, USA) at 25 °C.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were referenced to TMS. The high-resolution ESI mass spectrum was obtained on a Waters API X-TOF Ultima (Milford, MA, USA) instrument.

The stems and leaves of *L. leonurus* were collected from Montague Botanic Gardens, Cape Town, South Africa, and a voucher specimen (No. 6859) deposited at the herbarium of the Department of Botany, University of the Western Cape. Fresh leaves of the plant were washed, dried at 30 °C in a ventilated oven for 72 h, and then ground into fine powder of which 83 g was heated in methanol (3 L) under reflux for 24 h and then filtered. Excess solvent was removed *in vacuo* to afford a thick residue. Distilled water (1 L) was added to the residue and the solution was acidified using concentrated HCl. This acidified solution was extracted with chloroform (2  $\times$  250 mL), and then

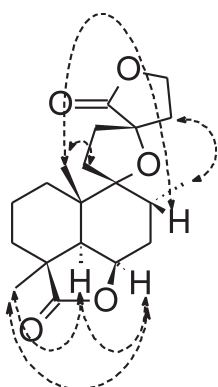


Figure 2 NOE correlations observed for compound 1.

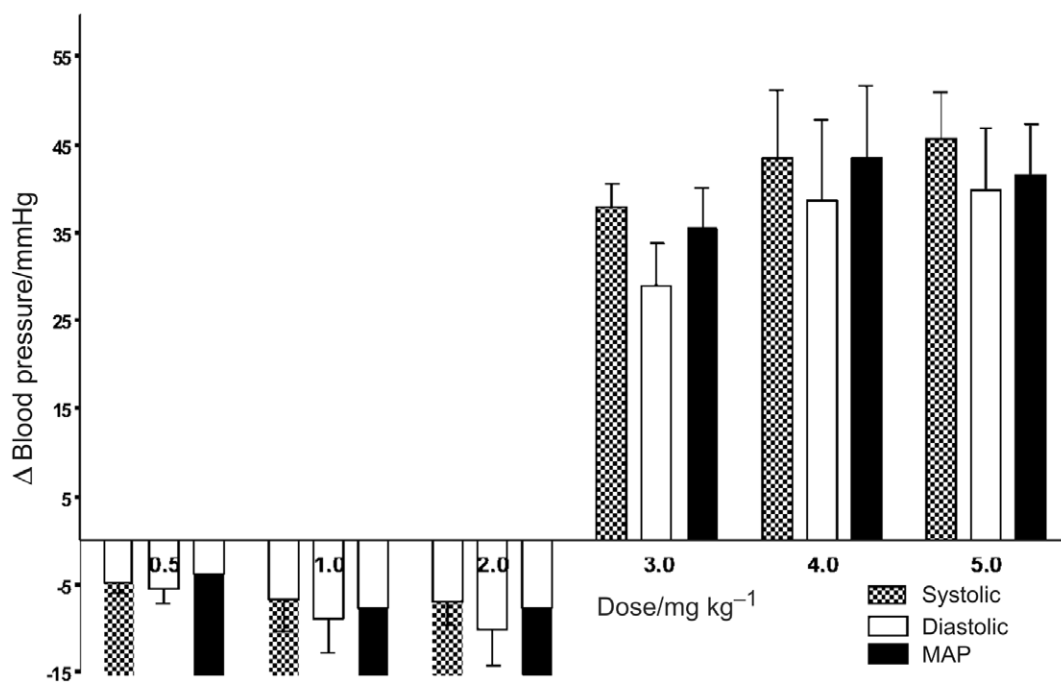


Figure 3 Effect of diterpenoid 1 on blood pressures (SP, DP, MAP) in anaesthetized normotensive rats.

basified with concentrated ammonia. The basified solution was in turn extracted using ethyl acetate ( $2 \times 250$  mL) and chloroform ( $2 \times 250$  mL). TLC was used to determine similar compounds in the two extracts which were combined, dried with anhydrous magnesium sulphate and evaporated. Chromatography of these residues was carried out on a silica gel (70–230 mesh grade) column, using ethyl acetate:hexane (1:4) as eluent. Further chromatographic purification on silica gel was carried out on the major fraction obtained using ethyl acetate:hexane:acetic acid as eluent (3:2:1 initially and then 3:2:0.5; and finally 3:1:0.5). Crystallization from chloroform yielded the pure diterpenoid 1 (1.526 g).

(13S)-9 $\alpha$ ,13 $\alpha$ -epoxylabda-6 $\beta$ (19),15(14)diol dilactone (1): colourless needles ( $\text{CHCl}_3$ ); m.p. 162.9–164.2 °C;  $[\alpha]_D^{19} +14.1$  (c 0.78,  $\text{CHCl}_3$ ); IR (Nujol)  $\tilde{\nu}_{\text{max}}$  2909, 1770, 1462, 1374, 1196, 1100, 994 and 911  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR ( $\text{CDCl}_3$ , 600 MHz)  $\delta$  4.70 (1H, dd,  $J = 4.6, 5.7$  Hz, H-6), 4.38 (1H, ddd,  $J = 6.0, 7.2, 9.0$  Hz, H-15a), 4.20 (1H, ddd,  $J =$

6.3, 7.2, 9.0 Hz, H-15b), 2.46 (1H, ddd,  $J = 6.3, 7.2, 13.3$  Hz, H-16a), 2.27 (3H, m, H-11a, H-12a, H-16b), 2.17 (3H, m, H-5, H-7eq, H-8), 2.09 (3H, m, H-1eq, H-3eq, H-12b), 1.90 (1H, ddd,  $J = 4.2, 12.2, 18.3$  Hz, H-11b), 1.73 (1H, m, H-2eq), 1.65 (1H, m, H-7ax), 1.56 (1H, m, H-2ax), 1.45 (1H, dt,  $J = 5.1, 14.4$  Hz, H-3ax), 1.28 (3H, s, H-18), 1.19 (1H, dt,  $J = 8.2, 13.2$  Hz, H-1ax), 1.05 (3H, s, H-20) and 0.91 ppm (3H, d,  $J = 6.1$  Hz, H-17);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 150 MHz)  $\delta$  183.8 (C, C-19), 171.4 (C, C-14), 93.5 (C, C-9), 83.6 (C, C-13), 76.1 (CH, C-6), 65.0 ( $\text{CH}_2$ , C-15), 45.8 (CH, C-5), 44.1 (C, C-4), 39.3 (C, C-10), 37.3 ( $\text{CH}_2$ , C-16), 34.3 ( $\text{CH}_2$ , C-12), 32.2 ( $\text{CH}_2$ , C-7), 31.5 (CH, C-8), 29.3 ( $\text{CH}_2$ , C-11), 28.3 ( $\text{CH}_2$ , C-3), 27.8 ( $\text{CH}_2$ , C-1), 23.8 ( $\text{CH}_3$ , C-20), 23.1 ( $\text{CH}_3$ , C-18), 17.9 ( $\text{CH}_2$ , C-2) and 17.5 ppm ( $\text{CH}_3$ , C-17); HRESIMS pos.  $m/z$  349.2009  $[\text{M} + \text{H}]^+$  (calcd. for  $\text{C}_{20}\text{H}_{29}\text{O}_5$  349.2015).

The anaesthetized normotensive rat model was used to determine *in vivo* cardiovascular activity.<sup>22</sup> Normotensive male Wistar rats, 250–400 g, 3–4 months old, were anaesthetized using

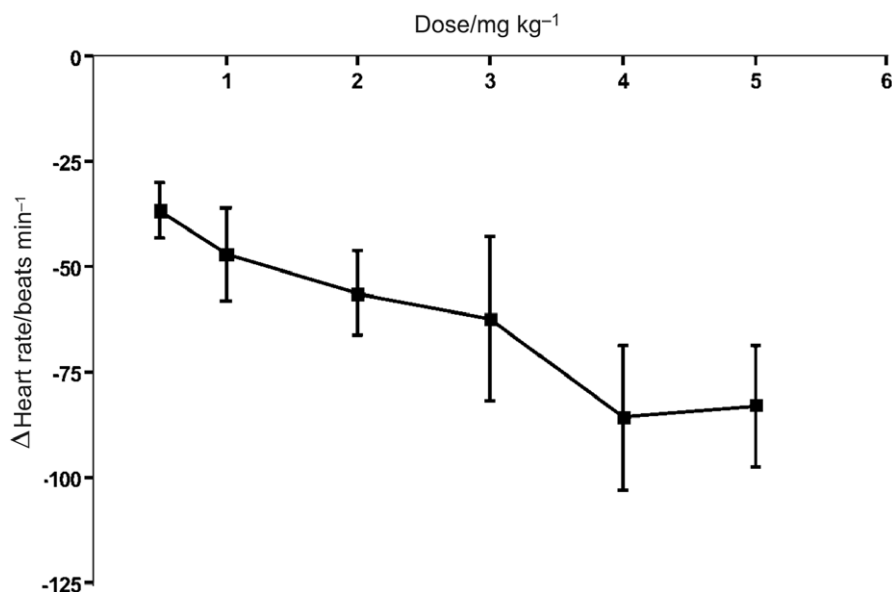


Figure 4 Effect of diterpenoid 1 on heart rate (HR) in anaesthetized normotensive rats.

sodium pentobarbital (30 mg kg<sup>-1</sup> I.P). Anaesthetized rats were tracheotomized to facilitate breathing and the external jugular vein and the femoral artery cannulated for the infusion of drug substances and for the measurement of blood pressure and heart rate respectively. Blood pressure and heart rate were monitored continuously throughout the experiment through a blood pressure transducer linking the arterial cannula to a PowerLab T20 (AD Instruments, Sydney, Australia) via a blood pressure amplifier (AD Instruments). Blood pressure readings measured as systolic pressure (SP), diastolic pressure (DP) and mean arterial pressure (MAP), and heart rate (HR) readings were recorded using the Chart 4.0 software (AD Instruments) running on a computer connected to the PowerLab T20. Randomized doses of the isolated compound (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg kg<sup>-1</sup>) were administered. Time intervals between successive injections were such as to allow for the return of blood pressure and heart rate to baseline values.

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